(Mini Review)

Colistin: A mainstay antibiotic for treatment of fatal Gram negative bacterial infections

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Abstract

Our world is facing an enormous disaster due to spreading of the life-threatening bacterial infections, especially after the spread of multidrug-resistant Gram-negative bacteria (MDR-GNB), including those resistant to known antibiotics such as fluoroquinolones, aminoglycosides, broad-spectrum penicillins, and other β-lactams antibiotics, such as monobactams and carbapenem. In addition, the a shortage of the production of new drugs that are able to overcome these life-threatening infections. This disaster has pushed the medical profession to re-use colistin, the old antibiotic, as the last hope drug for the treatment of these fatal infections. Colistin was discovered in 1950 and was rejected in most parts of the world due to its toxicity including nephrotoxicity. Unfortunately, the resistance against colistin has been discovered and this resistance has spread globally. Colistin resistance includes both intrinsic resistance and acquired resistance. The mechanisms of acquired colistin resistance were linked only to the chromosomal gene mutations until the discovery of the colistin resistance gene named the mobile colistin-resistant (*mcr*) gene which is mediated by the plasmid. In this review, we discuss the history, chemistry, spectrum of activity, mechanism of action, clinical uses and indications, adverse effects, mechanisms of colistin resistance, and antibiotic combinations with colistin.

Keywords: Antimicrobial resistance, *mcr* genes, Nephrotoxicity and Polymyxin.

Introduction

The increased resistance to antibiotics that launched among the GNB in the 1970s has become a serious global disaster [1]. The chief issue is that there is a shortage of probable alternatives that are capable of defeating life-threatening pathogens. Indicating that the resistance to the antibiotics could become a universal disaster that shows no signs of resolving [2]. Our world is facing a tremendous and growing threat from the rise of bacteria that are particularly resistant to nearly all existing antibiotics [2-4]. Antibiotic resistance has been referred to as "the silent tsunami facing modern medicine". The most urgent issue is the increasing incidence of life-threatening antibiotic-resistant Gram-negative (GN) bacterial infections. The rise of MDR-GNB and increasingly pan-drug resistant (PDR) strains has affected practice in every field of medicine [5, 6]. Unfortunately, the accomplished efforts in pharmaceutical manufacturing are not enough to resolve the bacterial resistance disaster. The shortage of newly approved antibiotics for these superbugs due to the deficiency in antibiotic discovery has pushed the medical community to reuse the older antibiotics that were limited in their use in the past, in particular, polymyxins, The reemerged colistin has been used mainly against fatal infections induced by MDR GNB [1]. Polymyxin antimicrobials are a structurally different class of cyclic non-ribosomal oligopeptides, which include (polymyxins A, B, C, D, and E), with polymyxin B and colistin (polymyxin E) being the only polymyxins available in the market [1, 7, 8]. Colistin has become the last resort antimicrobial is capable of defeating MDR GNB [9, 10].

1. Colistin:

1.1 History and discovery:

In Japan in 1949, Koyama discovered colistin from a flask of spore-forming soil bacterium, *Bacillus polymyxa* subsp. *colistinus* [11]. Colistin was utilized in both human and veterinary medicine [12, 13].

Colistin was gradually rejected in the 1980s from the majority of world regions due to its nephrotoxicity. Subsequently, treatment with colistin during the past two decades was restricted to the lung infections caused by MDR-GNB in cystic fibrosis (CF) patients [1].

1.2 Chemistry of colistin:

Colistin molecule consists of a cyclic cationic polypeptide attached through α-amide linkage to a chain of fatty acids. Its molecular weight is around 1750 Daltons. The amino acid constituents of colistin are L-Threonine, D-Leucine, and L-2, 4-Diaminobutyric acid (Dab). The chain of fatty acids may be 6-methyl-octanoic acid or 6-methyl-heptanoic acid for colistin A or colistin B, respectively [1].

The colistin antibacterial activity depends on its chemistry. The amino groups of the amino acid Dab at physiological pH are ionized, and thus colistin carries a net-positive charge, which represents a critical property to interact with the phosphate groups are present in the bacterial lipopolysaccharide (LPS) which is negatively charged. In addition, colistin possesses a chain of hydrophobic fatty acyl, which also can interact with the LPS and cell membranes [14].

Colistin has two forms available for the treatment of GN bacterial infections. The first is colistin sulfate (CS), which is used for topical and oral use. The second is colistimethate (colistin methanesulfonate sodium) (CMS)**,** which is a prodrug that is used by inhalation and parenteral routes. CMS has low toxicity when compared to CS [15].

1.3 Colistin spectrum of activity:

Colistin is bactericidal toward most GN aerobic bacilli, including *Escherichia coli, Acinetobacter baumannii*, *Klebsiella species, Pseudomonas aeruginosa*, and most of the *Enterobacter*iaceae family. However, colistin is ineffective against *Mycoplasma*, Gram-positive bacteria and other intrinsically resistant bacteria, such as *Morganella morganii,, Serratia marcescens*, *Providencia* spp*., Proteus* spp*., Vibro cholera, Brucella, Legionella, Campylobacter*, *Neisseria spp., Chromobacterium, Edwardsiella* spp*., Burkholderia cepacia*, some *Aeromonas* spp, anaerobic GN cocci, mammalian cells and eukaryotic microbes [16-18].

1.4 Colistin mechanism of action:

1.4.1 Direct antibacterial activity:

Colistin primarily destroys bacteria by disrupting their inner membrane (IM) and outer membrane (OM) as colistin has a detergent-like action, which increases the membrane's permeability. Colistin direct action occurs via a recognized model known as "self-promoted

uptake". In this model, the amphipathic property of colistin is vital for the colistin molecules uptake via the OM via the OM [16].

In the "self-promoted uptake" model, the cationic part of colistin, which include ionized Dab residues, primarily binds to the anionic phosphate groups of the lipid A moiety of the OM via electrostatic interactions. Colistin molecules then competitively displace the two divalent cations Ca^{+2} and Mg^{+2}, as colistin has a higher affinity toward LPS than Ca^{+2} and Mg^{+2} [13]. The attachment of colistin molecules to the LPS lipid A moiety and its insertion into the OM leads to the destabilization of LPS moieties and impairment of the OM, resulting in an increase in colistin uptake [1, 19]**.** The hydrophobic part of colistin insertion in the OM creates cracks in the membrane, which allows the "self-promoted uptake" process to occur [20]. Finally, it leads to lysis of the bacterial membranes and then leakage of the cytoplasmic and periplasmic contents and cell death. Remarkably, this process is independent of the entrance of colistin into the cell [10, 18].

1.4.2 Anti-endotoxin colistin activity:

Colistin has effective anti-endotoxin activity, as the lipid A fragment of the GNB LPS forms endotoxin. Colistin diminishes the endotoxin action by attaching and neutralizing LPS moieties. The *in vivo* value of this mechanism for the antibacterial activity is that it inhibits the ability of endotoxin to produce shock by releasing cytokines**.** Indeed, the exact mechanism of this suppressing action is still unclear [1, 21].

1.4.3 Vesicles contact pathway:

Colistin also produces antibacterial activity via a mechanism termed vesicle-vesicle contact. After crossing the OM, colistin attaches to phospholipid vesicles, resulting in the union of the inner part of the OM with the outer part of the cytoplasmic membrane. This fusion stimulates phospholipid exchange, causing phospholipid loss and resulting in osmotic imbalance and finally lytic cell death [16, 20].

1.4.4 Hydroxyl radical death pathway:

The pathway of hydroxyl radical involves the formation of reactive oxygen species, which induce oxidative stress. In general, the superoxide anion (O_2^-) is produced when colistin crosses the OM and IM. Following that, O_2^- is converted into hydrogen peroxide (H_2O_2). H_2O_2

then oxidizes ferrous (Fe^{2+}) into ferric (Fe^{3+}), inducing oxidative injury in the bacterial DNA, lipids, and proteins, which finally leads to cell death [13, 22].

1.4.5 Inhibition of respiratory enzymes:

It is a secondary mechanism of colistin action that occurs by inhibition of a vital respiratory enzyme, type II NADH-quinone oxidoreductase in the respiratory cycle. This mechanism has been identified in *E. coli, K. pneumoniae, and A. baumannii* [18, 23].

1.5 Colistin clinical uses and indications:

The Food and Drug Administration (FDA) approved colistin as an antimicrobial agent for treating infections caused by GNB. Colistin is active against various types of diseases, including Urinary tract infections (UTIs), eye infections, and ear infections. Colistin is also used for decontamination of the bowel [16, 24].

CMS is administrated by inhalation and intravenously in order to manage *P. aeruginosa* infections in CF patients [25]. Also, colistin has been used to cure bacteremia and ventilator-associated pneumonia caused by MDR GNB [26].

Moreover, CMS is effective in the ventriculitis treatment that is caused by MDR *A. baumannii* [27]. In veterinary medicine, polymyxins are given to animals on almost all continents, mainly to treat digestive disorders in addition to growth promotion [28].

1.6 Colistin adverse effects:

Colistin produces toxicity, such as nephrotoxicity and neurotoxicity, as well as neuromuscular blockage, which sometimes leads to death. Consequently, colistin usage was limited in the 1980s, except for the treatment of CF infectious disease [29, 30].

The nephrotoxicity was noticed to be prevalent in patients over 60 years old and is associated with a low glomerular filtration rate. Therefore, monitoring kidney function in patients receiving colistin is very important [31]. Higher colistin doses are proportional to the high rate of nephrotoxicity [32]. However, in recent studies, the nephrotoxicity incidence is less frequent and less severe if compared to the old studies. Additionally, the colistin neurotoxic outcome is usually not severe and resolves after immediate cessation of the drug. Moreover, neuromuscular blockage and apnea cases were not present in the latest literature [10, 33].

1.7 Mechanisms of colistin resistance:

Generally, GNB can evolve resistance against colistin intrinsically or by adaptation mechanisms that include chromosomal gene mutation and horizontally acquired resistance genes [34, 35]. Colistin has cross-resistance with polymyxin B [1, 19].

1.7.1 Intrinsic resistance:

Natural resistance to colistin is present in many organisms, and those organisms have been used to understand the mechanisms of colistin resistance besides its mode of action [36, 37]. Bacterial species that possess intrinsic resistance to colistin include *Providencia spp., Proteus spp., Neisseria spp., Burkholderia cepacia,* and other species [17].

The polymyxins attach and penetrate the phosphatidylethanolamine (PE) monolayers but do not affect methylated PE as in *Proteus* species. Methylated PE has been confirmed by the phenotypic change of polymyxin B resistant *Proteus mirabilis* to become sensitive by using a sulfadiazine medium, this change may be due to the blockage of the synthesis of the methylated PE [36].

Another study stated that resistance to polymyxins in *Serratia marcesens* and *P. mirabilis* is due to bacterial LPS modification through cationic substitution. This is linked to *arnBCADTEF* operon expression and the *eptB* gene. After their expression, the phosphoethanolamine (PEtN) and/or the 4-amino-4-deoxy-L-arabinose (L-Ara4N) cationic groups are added to the bacterial LPS, this addition leads to a decrease in the bacterial LPS negative charge which prevents colistin attachment to the bacterial LPS and subsequently prevents colistin action [38-40]. The presence of *RppA*/rppB two-component system (TCS) has also been discovered to play a role in the *arnBCADTEF* operon activation [41, 42]. Studies on *S. marcescens arnBCADTEF* operon have revealed that *arnC* and *arnB* mutants showed reduced resistance to colistin. The minimum inhibitory concentration (MIC) of the *arnC* and *arnB* mutants was reduced from 2,048 to 2 μg/ ml [43]. In addition, a putative acetyltransferase is present in *P. mirabilis,* which also takes a part in the addition of L-Ara4N to lipid A part of the bacterial LPS [44].

1.7.2 Acquired resistance:

The polymyxins acquired resistance mechanisms may be plasmid-mediated or chromosomally mediated. Resistance to colistin has been exclusively associated with chromosomal gene mutation until 2015 [45, 46]. Resistance mechanisms mediated by the chromosomes can be briefly described as follows: (I) LPS modifications via cationic moieties. (II) LPS losses. (III) Efflux pump systems overexpression. (IV) Capsular overproduction in some GNB**.**

I. LPS modification enzymes:

The main strategy that permits the GNB to overcome the bactericidal activity of colistin depends on making changes in the LPS of the GNB, by decreasing its negative charge, thus prohibiting colistin binding and consequently colistin action [18, 47]. This could be gained by substituting the phosphate groups in the LPS with the cationic groups L- PEtN and/or Ara4N [13, 16]. The expression of nearly all genes that modify the LPS is under the control of various TCS [48].

Two of the most broadly studied TCS are the PhoP/PhoQ (PhoPQ) and PmrA/PmrB (PmrAB) systems whose regulations and functions were found to be overlapped **[15].** PmrA/PmrB and PhoP/PhoQ TCSs both have a sensor kinase, that can sense the signals of the environment, such as the decrease in Mg^{+2} and Ca^{+2} ions of the OM, thus the presence of colistin, then they alter the TCSs expression pattern, that modulates the expression of colistin resistance genes [49, 50]. The upregulation of TCSs by mutations in their regulatory systems results in colistin resistance by extra cationic moieties in addition to the LPS [48]. The PhoQ protein of the PhoP/PhoQ TCS acts as a repressor of transcription of PhoP activity. Mutations in PhoQ permit the PhoP to induce expression of *arn* operon leading to colistin resistance. This clarifies that the inactivation of PhoP by mutations completely restores the activity of colistin [48].

Genes that modify bacterial LPS involve the *pmrCAB* operon, which encodes for 3 proteins: the PmrB, the sensor kinase, the PmrA, the response regulator, and the PmrC, the PEtN phosphotransferase. The PmrB protein is a sensor tyrosine kinase that stimulate the response regulator, PmrA. PmrA then triggers the transcription of PmrC, which works as PEtN phosphotransferase, which is responsible for the PEtN cationic compound addition to bacterial LPS [18, 49, 50].

The *pmrE* gene, in addition to the *pmrHFIJKLM* operon, also takes part in the bacterial LPS modification. They participate in the L-Ara4N moiety synthesis and its adherence to the lipid A part of the bacterial LPS [49]. The *mgrB* gene encodes a small regulatory protein, which decreases the PhoQ kinase activity. Inactivated *mgrB* gene led to up-regulation of PhoP/PhoQ expression and consequently increased the colistin resistance [51, 52] In *P. aeruginosa*, there are several TCSs including ParR/ParS, CprR/CprS, ColR/ColS as well as PhoPQ and PmrAB. These TCSs activate the *arnBCADTEF* operon which leads to bacterial LPS modification [17, 53, 54].

A study on *pmrCAB* and *lpxACD* operons that present in colistin susceptible *A. baumannii* isolates, revealed the possibilities of mutations in both operons without development of colistin resistance. This alerts us to the significance of interpreting mutated operons about susceptible colistin isolates of the identical sequence type and global clones (ST/GC) content [55].

A study on *Aeromonas hydrophila* revealed a novel mechanism for colistin resistance. These mechanisms were able to generate low to intermediate-level colistin resistance. First, EnvZ/OmpR TCS has the ability to upregulate the *arnBCADTEF* operon expression, this mediates the bacterial LPS modification. Second, EnvZ/OmpR TCS regulates the auto-transporter gene3832 expression, which was able to decrease the OM permeability as a response to the presence of colistin. Third, the removal of envZ/ompR TCS stimulates the PhoP/PhoQ TCS, which also takes a part in the development of colistin resistance. Fourth, the *mlaFD173A* gene mutant provides a high level of colistin resistance via the upregulation of the *Mla* gene pathway, which is an OM lipoprotein-encoding gene [56].

Another mechanism for colistin resistance in *E. coli* was attributed to RpoE stress system, which is able to mediate the resistance against colistin without affecting the lipid A [57]. Also, a degrading colistin protease discovery revealed the diversity of resistance mechanisms against colistin [58].

An important resistance pattern is polymyxin heteroresistance. heteroresistance is the result of different responses within the same population of bacterial cells to antibiotics. This poorly understood phenomenon complicates the investigations of antibiotic resistance. The heteroresistance phenomenon occurs when a small bacterial subpopulation has different degrees of polymyxin susceptibility [59, 60]. Resistance to polymyxins can be gained phenotypically by

polymyxin-heteroresistant bacteria. The MIC in the majority of these bacteria is ≤ 2 mg/l, while these subpopulations are capable of surviving at polymyxins concentrations>2 mg/l. The heteroresistance frequency against polymyxin in *P. aeruginosa* is rare, but it has been found in MDR *A. baumannii and K. pneumonia* [16].

Heteroresistance to polymyxins was suspected to be due to mutations in chromosomal genes, such as lipid A biosynthesis genes, *lpxA*, *lpxC,* and *lpxD,* or by the addition of L-Ara4N [61, 62]. It also can be caused by mutations in PmrAB TCS in *A. baumannii* [63].

II. LPS Loss

LPS loss was discovered in *A. baumannii.* This effect occurs by inhibiting the biosynthesis of lipid A through *lpxC, lpxA,* and *lpxD* gene mutation. These mutations lead to complete lipid A loss, which prevents the polymyxins OM interaction and subsequently colistin activity [62].

III. Efflux pumps

Generally, the efflux pump activation results in the development of resistance to various antibiotics including, colistin. Different efflux pumps are present in bacteria, such as the Sap (sensitive antimicrobial peptides) proteins, KpnEF, the AcrABTolC complex, BrlR has been detected. The Sap proteins contain five different proteins which are encoded by the sapABCDF operon [45]. The efflux pumps systems MtrC-MtrD-MtrE, KpnEF, AcrAB-TolC, RosAB, VexAB, and NorM have been also defined to allow tolerance of bacteria against polymyxin B, but these pumps influence polymyxin tolerance in a small number of cases [64]. In addition, the efflux pumps AcrAB confer polymyxin resistance to *K. pneumoniae* [18, 65]. The mutations in AcrAB and kpnEF, efflux pumps, have been reported to be able to decrease the colistin MIC by 2-fold and increase survival at low polymyxin concentrations [18].

The attention received to efflux pumps' role in the development of resistance to colistin is less than other resistance causes. The inhibitors of the efflux pump 1-(1-naphthylmethyl)-piperazine (NMP) and Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) have the ability to reverse the colistin resistance, which suggests that the importance of the efflux pumps role in the development of colistin resistance [66, 67]. Overexpression of efflux pumps in addition to LPS modification and their regulators are important mechanisms of the resistance against colistin in *mcr*-negative *K. pneumoniae* [68].

IV. Capsular overproduction

Capsular overproduction mechanisms as in *Klebsiella* spp. involve the capsule synthesis regulator, this regulator modifies the expression of the capsular polysaccharide biosynthesis, leading to an increase in capsule production that hides the site to which polymyxin bind and thus confers resistance to colistin. The capsule synthesis regulator also regulates PmrA/PmrB and PhoP/PhoQ TCSs and it thus indirectly modifies the bacterial LPS **[69].**

In China, the first colistin resistance gene mediated by plasmid was found in 2015. This gene was designated as a mobile colistin resistance (*mcr-1)* gene. It was identified in *E. coli* isolate. The *mcr-1* gene has become a significant cause for the spread of colistin resistance between several GNBs and helped in explaining the unknown colistin resistance mechanisms **[35].**

After its first discovery, the *mcr-1* dissemination among several *Enterobacter*iaceae species was detected. Worldwide, the *mcr-1* gene has been reported in over 30 nations in five continents **[2, 70-72].** It has been found in several isolates from farms, wild animals, foods, humans, aquatic environments, and hospital sewage **[47, 48, 73].** The *mcr-1* gene has been experimentally transferred in the lab from *E. coli* to *P. aeruginosa* **[48, 70, 74].** The *mcr-1* gene dissemination worldwide proposes that veterinary use has probably sped up its dissemination. This is in agreement with the hypothesis which stated that: livestock is the primary cause of *mcr-1* dissemination **[71].**

The *mcr-1* gene activity leads to an increase in the MIC of colistin by 4-8 fold, which proves that the *mcr-1* gene alone, without any other resistance genes, is sufficient to produce resistance against colistin **[18].** MCR-1 protein is a PEtN transferase enzyme that belongs to the alkaline phosphatase superfamily “YhjW/YjdB/YijP” **[74].** The MCR-1 protein leads to the addition of PEtN molecule to the lipid A of the bacterial LPS, this leads to an increase in the positive charges on the bacterial LPS and subsequently reduces the colistin binding to the bacterial LPS **[2, 19, 35, 47].** The MCR-1 amino acid sequence analysis revealed that MCR-1 is related to the pmrC, the PEtN transferases, which are present in *Paenibacillus* spp. **[47].** Also,

MCR-1 is similar to the PEtN transferases, from *Campylobacter jejuni* and LptA from *Neisseria meningitidis* **[35, 75].**

The MCR-1 also interacts with bacterial membrane proteins, a major six proteins were identified, including the stress response proteins SspB (stringent starvation protein B) and DnaK (chaperone protein), ribosomal proteins (RpsE, RpsJ, and RpsP), and the transcriptional regulation protein H-NS were found to be a site of MCR-1 protein interaction, these membrane proteins were identified in *E. coli* strains including, *E. coli* BL21 (DE3) (pET28a-*mcr-1*), *E. coli* DH5α (pUC19-*mcr-1*), and *E. coli* BL21 (DE3) (pET28a-*mcr-1-200*). These interacting proteins with MCR-1 were primarily involved in RNA degradation and ribosome activity. In addition, the AcrA and TolC, efflux pumps also take a part in MCR-1 interaction. This proves that the efflux pumps involved in promoting colistin resistance which mediated by *mcr-1* gene [76]

The transferable resistance genes of colistin have extended more away from the *mcr-1* gene to include many novel alleles. Nine alleles for the *mcr-1* gene have been reported, including; *mcr-2* **[75],** *mcr-3* **[77],** *mcr-4* **[78],** *mcr-5* **[79],** *mcr-6* **[80],** *mcr-7* **[81],** *mcr-8* **[82],** *mcr-9* **[83],** and the recently detected *mcr-10* **[84]**

Even though all alleles of *mcr* have been categorized as gene encoding for PEtN transferases, they have commonly conserved amino acid moieties and they share variable similarity degrees in their amino acid sequences. Thus this amino acid variability signifies different origins of the resistance gene **[79].** Investigations of the MCR protein sequences revealed that the MCR-1 protein has amino acid sequence similarity with, MCR-2 (81%), MCR-3 (34%), MCR-4 (33%), MCR-5 (31%), MCR-6 (82%), MCR-7 (29%), and MCR-8 (31%) **[85].** Three-dimensional (3D) structural models of MCR-1 to MCR-9, presented that MCR-3, 4, 7, and 9, are having a great degree of similarity at their structure level **[83].**

The most prevalent *mcr* gene among *Enterobacter*iaceae is the *mcr-1* **[35, 48, 71].** The *mcr-2* gene was detected in *E. coli* isolated from cattle and pigs in Belgium **[75]**. The *mcr-3* gene was identified in an *E. coli* isolate from a swine specimen in China **[77]**. The phylogenetic analysis revealed that the *mcr-3* gene is different from *mcr-1* but *mcr-3* is related to PEtN produced from *Aeromonas* spp. **[47]**. Sequence alignment recommended that the *mcr-3* gene has

a higher similarity to the *EptA* gene (53.1%) than to *mcr-1* (44.1%) **[15]**. The *mcr-3* gene encodes for a weak form of *MCR*-like enzyme and its presence with *mcr-1* gene does not give a considerable additive on colistin resistance **[70]**.

The *mcr-4* gene was first identified in *Salmonella enterica* in Italy isolated from a specimen of a pig **[78, 86]**. Amino acid sequence analysis represented that the MCR-4 has similarity to MCR-1 (33%), MCR-2 (35%), and MCR-3 (49%) **[47].** The *mcr-5* gene was first identified in Germany in *S. paratyphi B* isolated from poultry **[79].** Protein analysis revealed that it has sequence homology with MCR-1 (36.11%), MCR-2 (35.29%), MCR-3 (34.72%), and MCR-4 (33.71%) **[15].** The *mcr-6* gene was discovered in *Moraxella* spp. which was present in pig isolates from Britain **[80].** The *mcr-7* gene was discovered in *K. pneumoniae* which was isolated from chicken birds in China **[81]**.

The *mcr-8* gene was discovered in *K. pneumoniae* **[82]**. The *mcr-9* gene was isolated from *Salmonella typhimurium*. Examinations of *mcr-9* genetic environment has revealed that the MCR-9 amino acid sequence is closely related to those of MCR -3 and MCR -7 **[83]**.

The novel *mcr-10* gene was discovered in China in *Enterobacter roggenkampii*. The novel MCR-10 identity of amino acid with MCR-1 (29.31%), MCR-2 (27.09%,), MCR-3 (61.60%), MCR-4 (42.49%), MCR-5 (28.94%), MCR-6 (26.53%), MCR-7 (58.26%), MCR-8 (35.81%), and MCR-9 (82.93%) **[84]**.

The *mcr-1* gene was discovered in an IncI2 plasmid termed pHNSHP45 (64015 bp) **[35, 48, 71].** After that, many *mcr-1* harboring plasmids have been identified, which belong to various groups with different sizes ranging between 58 and 251 kb **[18],** including IncHI2 **[87]**, IncX4 **[74]**, IncP **[73]**, IncY, IncK2, IncF, IncFIB, IncFI, IncFII **[70]**, IncQ, and IncN **[71]** plasmids. Two different types of plasmids harboring the *mcr-1* gene have been confirmed to occur together in an *E. coli* isolate, such as the pGD65-4, IncI2, and pGD65-3 plasmids **[72].**

1.8 Colistin in combination with other antibiotics:

A synergistic effect has been determined *in vitro* from colistin combination with carbapenems in carbapenem-resistant GNB [88]. Also, there was evidence of synergy with imipenem against *A. baumannii* [89-91], *P. aeruginosa* [92], and *Enterobacter cloacae* [93]. In addition, synergy was detected against *A. baumannii*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*

with doripenem [94-96]. Moreover, synergy with rifampin and ceftazidime against *P. aeruginosa* and *A. baumannii* has been submitted [97-100]. Other studies verified significant synergy between colistin and glycopeptide against *A. baumannii* [101, 102].

Sundaramoorthy *et al*. approved the activity of ursolic acid, which potentiated the colistin bactericidal effect. Ursolic acid reduced the bacterial bioburden in combination with colistin, by 1–1.58 log fold. Mechanistic explorations revealed that the colistin efflux was inhibited by the action of ursolic acid. Also, ursolic acid enhances OM permeability, which plays a role in the facilitation of colistin attack on OM and IM. Ursolic acid when tested in zebrafish it was non-toxic [67]

In the study of Zhou *et al*., they checked the activity of osthole compound against colistin-resistant *E. coli* and *K. pneumoniae*, *in-vitro*, in addition to *in-vivo*, in an infection model of a mouse. The bacterial strains used harbored *mcr-1* gene. The reported results showed promising results in overcoming the colistin resistance. The activity of combination was capable of preventing the bacterial growth rather than the individual compounds [103]

In the Hanpaibool *et al*. study, they tested 4 different pyrazolone compounds *in vitro* against *E. coli* strain that harbors *mcr-1* gene. They stated that the pyrazolones compounds were found to be effective in lowering the MIC of colistin in the *mcr-1* harboring colistin-resistant *E. coli* strain [104]

The saturated fatty acids (SFAs) such as sodium caprate (SC), significantly potentiate the colistin activity toward GNB harbor *mcr* genes. Colistin and SFAs together efficiently inhibit the biofilm formation and elimination of matured biofilms. Mechanistically, the SFAs addition to colistin, reduce bacterial LPS modification by encouraging LPS biosynthesis, as well as inhibiting the MCR enzyme activity. This combination was tested *in vivo* in animal models infected by *mcr*-positive GNB and it exhibited an effective result [105].

Curcumin and colistin delivery in liposomes encapsulating the combination (Lipo-cc). The Lipo-cc antibacterial activity against colistin-resistant GNB was confirmed, which was more effective than the mono curcumin and colistin compounds. Mechanistically, the Lipo-cc was able to restore the colistin affinity for the bacterial membrane. In addition, lipo cc improves the curcumin uptake, which affects the efflux pump leading to a reduction in its activity. The lipo-cc does not show any toxicity and its therapeutic efficacy was confirmed in an infection model [106].

The disulfiram (DSF), the alcohol-abuse drug, has a powerful antibiotic adjuvant activity, which enhances the carbapenems as well as colistin activity toward New Delhi metallo-β-lactamase (NDM) and MCR-producing GNB. Mechanistically the studies show that DSF improves colistin activity as DSF has the ability to increase the harmful action of colistin on cell membranes, in addition to disruption of the metabolism of GNB. The synergistic efficacy was evaluated in animal models and the combination has effectively cured MDR GN bacterial infections *in vivo* [107].

Selenium nanoparticles and colistin combination were tested *in-vitro* on PDR *A. baumannii* to test their activity together. The combination has a strong synergistic effect against colistin-resistant *A. baumannii* [108].

A nanocomplex of CMS with guanidinium polymer pEt_20 nanopolymer (NP) (CMS-pEt_20 NP) was developed to reverse the resistance against colistin. The CMS-pEt_20 NP enables colistin resistance reversal in addition to, full eradication of *mcr*-positive GNB. The mono-treatment at equivalent doses with polymer or colistin didn't exhibit antibacterial activity. Mechanistically, studies revealed that the CMS-pEt_20 NP improves CMS affinity for the altered GNB membrane that is resistant to colistin. This revives the damaging property of colistin to cell membrane. The improved membrane permeability, which is caused by CMS, promotes pEt_20 influx which generates intracellular reactive oxygen species stress, that results in eliminating the colistin-resistant GNB. More importantly, the synergistic efficacy was evaluated in infected animal models with complete mouse survival. Furthermore, the nanocomplex is safe both *in-vitro* and *in-vivo* [109].

Gallium nitrate (GaNt), one of the antimicrobial candidates, shows a potentiating effect on the activity of colistin toward clinical isolates of MDR *K. pneumoniae*. This significant increase in colistin antimicrobial activity was confirmed *in-vitro* and *in-vivo* using a murine lung infection model. Mechanistically, GaNt represses the antioxidant activity in the bacteria, in addition to increasing the intracellular accumulation of ROS in bacteria, this action was enhanced by colistin [110].

Gigantol, a bibenzyl phytocompound, was tested for restoring the sensitivity of *mcr* harboring GNB. The activity of colistin and gigantol combination against MDR GNB was studied *in-vitro* and *in-vivo*. Gigantol was able to restore the colistin activity against *mcr* harboring GNB including, *E.coli, Salmonella,* and *K. pneumoniae* that carry *mcr-1, mcr-3,* and

mcr-8, respectively. Mechanistically, gigantol was able to down-regulate the *mcr* genes expression, and subsequently decrease the MCR-1 protein production, and stop its action by attaching to residues of amino acids, Pro481 and Tyr287. Evaluation of gigantol safety presented that the gigantol addition was able to relieve the colistin hemolysis effect [111].

Conclusion

The world is encountering a huge and rising threat from the appearance of bacteria that are practically resistant to all existing antibiotics. Dissemination of MDR GNB may be silent and poses significant challenges for infection control measures. The most serious GN bacterial infections that occur in the healthcare environment are most commonly produced by *Enterobacteriaceae* in addition to *Pseudomonas aeruginosa* and *Acinetobacter* species. MDR GN bacterial pathogens are increasingly spread in the community.

Colistin (polymyxin E), a non-ribosomal cyclic oligopeptides antimicrobial agent, was considered “a miracle” antibiotic in the 1950s at its first commercial, with low resistance level and bactericidal activity toward GNB. After the colistin discovery, it was used in Japan, Europe, and in the United States during the 1950s **[112].** Colistin was gradually rejected in the early 1980s in many parts of the world due to its nephrotoxicity. Subsequently, colistin use was limited to the lung infection treatment in patients with CF during the previous two decades **[1].** The development of the bacterial resistant strains to mainly all available antibiotics and the lack of novel antimicrobial agents that are able to defeat these life-threatening pathogens have led to the reevaluation of polymyxins, particularly colistin, as a last hope drug for the treatment of these infections **[10, 19].**

Unfortunately, colistin reuse especially for the treatment of carbapenem-resistant pathogens, has led to the existence of colistin resistance. Acquired colistin resistance mainly results from chromosomal gene mutations, but, the discovery of *mcr-1* to *mcr-10* genes which carried on plasmid, encoding a phosphoethanolamine transferase, also aided in the development of colistin resistance.

- **Conflict of Interest**

  There are no conflicts of interest

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